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MOSQUITO VECTOR BIOLOGY AND CONTROL IN LATIN AMERICA—A 20TH SYMPOSIUM

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ABSTRACT. The 20th Annual Latin American Symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 76th Annual Meeting in Lexington, KY, in March 2010. The principal objective, as for the previous 19 symposia, was to promote participation in the AMCA by vector control specialists, public health workers, and academicians from Latin America. This publication includes summaries of 40 presentations that were given orally in Spanish or presented as posters by participants from 5 countries in Latin America, the United Kingdom, and the USA. Topics addressed in the symposium included: surveillance, chemical and biological control, and insecticide resistance associated with *Aedes aegypti*; distribution, behavior, and control of *Culex*; bionomics, ecology, and chemical and biological control of *Anopheles* vectors of malaria; insecticide resistance; and studies of dengue, West Nile virus, and *Triatoma*.

KEY WORDS Mosquitoes, dengue, malaria, West Nile virus, surveillance, chemical control, biological control, bionomics, insecticide resistance, *Aedes*, *Anopheles*, *Culex*, and *Triatoma*

INTRODUCTION

The American Mosquito Control Association (AMCA) is dedicated to the study and control of mosquitoes, other arthropods, and vectors and promotes cooperation and interaction among professionals and students in this field both in the USA and internationally. To promote greater and more active participation among a portion of its international membership, a Spanish language symposium was held first at the AMCA Annual Meeting in 1991 and at all subsequent meetings. In addition to providing a forum for scientists whose first language is Spanish, the session promotes interaction with mosquito control industry representatives; with professional colleagues in the USA who are involved in mosquito vector control, training, and research at the university level; and with state and federal government officials.

This publication includes summaries of 40 presentations that were given in Spanish by participants from 5 countries in Latin America, the United Kingdom, and the USA. Topics addressed in the symposium included surveillance, chemical and biological control, and insecticide resistance associated with *Aedes aegypti* (L.) and dengue viruses; distribution, behavior, and transmission of West Nile virus, and control of *Culex*; bionomics, ecology, and chemical and biological control of *Anopheles* vectors of malaria; insecticide resistance; and studies of *Triatoma*. Summaries of 17 previous symposia have been published (Clark and Suarez 1991, 1992, 1993; Clark 1995, 1996; Clark and Rangel 1997, 1998, 1999; Clark et al. 2000; Clark and Quiroz Martinez 2001, 2002, 2004, 2005; Clark and Rubio-Palis 2006, 2007, 2008, 2009).

SUMMARIES

Time and cost comparisons of total counting and rapid sweeping estimations for *Aedes aegypti* pupae surveillance

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Aedes aegypti pupae surveillance, based on total counts is time consuming and, therefore, expensive and impractical. We developed a simple, rapid, and robust sweeping method to accurately estimate pupal numbers in their most productive habitats, large domestic water-storage containers. In this study, we performed time comparisons between the sweeping method and total counts. The average time to estimate and count 200 pupae in a 220-liter drum, a <1,000-liter tank (GT1), and a >1,000-liter tank (GT2) per container type and water level (1/3, 2/3, and 3/3) (repeated 10 times) was then used to estimate the times and costs of field-surveillance data of 329 water-storage containers distributed in 319 houses. In semifield and field conditions, the sweeping method was 9.0 and 5.2 times, 5.0 and 5.5 times, and 3.0 and 3.0 times faster than total counts for GT2, GT1, and drums, respectively, with an average of 4 times faster. The sweeping method took 1 day to survey the 329 pupae-positive containers, at an estimated cost of US $132, compared to 4 days, at an estimated cost of US $528 using total counts. We, therefore, strongly

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recommend using our sweeping method for *Ae. aegypti* surveillance and control programs.

Evaluation of teneral *Aedes aegypti* emerged from four artificial containers

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We examined the relationships among body size, pregravid stage, and concentration of carbohydrates from teneral mosquitoes emerged from cement tanks, plastic or metal drums, flowerpots, and tires. Pupae were collected in each container; after females emerged, wing-length was measured as an index of mosquito size. The anthrone method was used to determine the amount of glycogen, sucrose, and trehalose in female mosquitoes. In total, 166 females were dissected. From these, 48 and 118 were classified as pregravid and gravid, respectively. The results suggest that small mosquitoes present mostly the pregravid state compared with bigger mosquitoes ($t = 8.009$, $P < 0.0001$). The trehalose level was directly associated with the largest male and female of *Aedes aegypti* ($r^2 = 0.95$, $P = 0.185$ and $r^2 = 0.216$, $P = 0.038$, respectively). The pregravid stage was mostly frequent in small mosquitoes, suggesting that multiple feeding is a common behavior for mosquitoes of smaller size, but not so frequent in large *Ae. aegypti*.

Cement tank: An artificial water container for *Aedes aegypti*

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An entomological survey was conducted in 200 houses in the community of Huixtla, Chiapas. The relative importance of diverse *Aedes aegypti* oviposition sites was determined and house and container index were used as indicators of entomological risk for each class of container. In total, 13,003 artificial containers were reported; of these, 77% (10,011) and 23% (2,992) were found outside and inside the houses, respectively; these data reveal the existence of 3.34 times more containers outside than inside the houses ($\chi^2 = 57.20$, $P = 0.0001$). When the frequency per container was obtained, the cement tanks were the third (14%) and fifth (3%) containers with the most abundant larval population inside and outside the houses, respectively. However, this class of container occupied the first place of those containers with water inside (96%) and outside (91%) the houses, respectively. The tanks showed a house index of 45 and 29, inside and outside the house, whereas container index was 38 and 13 inside and outside of houses, respectively. These findings suggest that cement tanks are important breeding sites because they are preferred by mosquitoes and are abundant in Huixtla, Chiapas.

Toxicity of spinosad and temephos against mosquito larvae in a tire cemetery in Allende, Nuevo Leon, Mexico

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Toxicity of spinosad and temephos was evaluated in a tire cemetery in Allende, Nuevo León. In total, 30 tires were used in this evaluation. Groups of 10 were used for each larvicide and a control. Samples were taken weekly after treatments application. Statistical difference was found among treatments. Mosquito larvae were found only in the control treatment.

Results using different formulations of Natular™ (spinosad) in Kentucky

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The active ingredient of Natular™ (spinosad) is a product derived from a naturally occurring soil bacterium. Spinosad represents a unique chemical class and mode of action different from all other existing larvicides. During Kentucky's 2008 and 2009 season, several evaluations were designed to measure efficacy and performance of Natular™. Results indicate efficacy against an array of mosquito species, including *Aedes japonicus* Theobald, *Aedes triseriatus* Say, *Aedes trivittatus* Coquillett, and *Anopheles quadrimacu-*

latus Say. Species susceptibility was achieved at label rates in several habitats, including abandoned swimming pools.

Evaluations of the new pyriproxyfen slow-release resin against wild strains of *Aedes aegypti* in semifield conditions

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We tested 2 different pyriproxyfen formulations (granules and slow-release resin: 50 ppb; manufacturer's suggestions) in 250-liter drums under semifield conditions. For this study, 2 liters of water were collected from these containers for laboratory analyses on days 0, 2, 4, and 7 and subsequently every 7 days until day 70 after treatment. For these analyses, food and 25 early 4th-stage *Aedes aegypti* L. of either the reference (LSHTM Gower) strain or 2 strains from dengue virus endemic areas (Los Olivos and Soledad) were added to four 250-ml replicates of each water-treated formulation, and the emergence inhibition (% EI) was determined after 7 days. In this study, water from the pyriproxyfen granule–treated drums inhibited the emergence of all of these *Ae. aegypti* strains by greater than 50% for over 1 month (day 36), and there were no significant differences between their susceptibilities to this hormone analogue. In contrast, the slow-release resin formulation showed greater than 50% EI of these mosquito strains for a period of only 4 days after treatment. These results suggest that the dosage of the pyriproxyfen slow-release resin formulation must be increased for effective *Ae. aegypti* control in their principal oviposition sites (water-storage tanks and drums).

Resistance to deltamethrin and enzymes associated with *Aedes aegypti* from western Venezuela

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Susceptibility and resistance mechanisms of adult mosquitoes to deltamethrin in 4 populations of *Aedes aegypti* collected from 3 states of Venezuela were determined. Bioassays were carried out using the bottle assay and the biochemical assay in microplates. New Orleans (NO) strain was used as reference. Results showed resistance to deltamethrin in 3 populations: Tres Esquinas (resistance ratio [RR] = 8.06), Pampanito (RR = 6.9), and Lara (RR = 9.05). Ureña proved susceptible, with RR = 3.4. Glutathione-S-transferase (GST) exceeding the resistance threshold established by NO strain was found in females from Lara and Ureña, suggesting cross-resistance with DDT (dichlorodiphenyltrichloroethane). Pampanito showed increased levels of β esterases, GST, and insensitive acethylcholinesterase (iAChE), while Tres Esquinas populations showed all elevated enzymes, with the exception of GST, in comparison with NO strain, suggesting resistance to different insecticide groups.

Knockdown resistance mutation (VAL1016) in *Aedes aegypti* from Mexico

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Pyrethroids are commonly used as mosquito adulticides, and the evolution of resistance to these compounds is a major threat to public health. "Knockdown resistance" to pyrethroids (KDR) is frequently caused by nonsynonymous mutations in the voltage-gated sodium channel transmembrane protein (para), which reduce pyrethroid binding. Early detection of KDR is critical to the development of resistance management strategies in mosquitoes including *Aedes aegypti*, the most prevalent vector of dengue and yellow fever viruses. Earlier studies have shown 7 novel mutations in hydrophobic segment 6 of domain II of para in *Ae. aegypti*. We found 2 new mutations never detected in Latin America in these same codons. A transition in the first position of codon 1011 encodes a Val replacement, while a transition in the first position of codon 1016 encodes an Iso replacement. The present study analyzed the rise of KDR mutation in 14 states of Mexico over the past 7 years and determined that the mutation KDR has been increasing considerably, mainly in the state of Veracruz, where populations from Martinez de la Torre, Tantoyuca, and Poza Rica did not present frequencies of KDR in 2000. For 2007, the frequencies increased to 0.43, 0.38, and 0.70, respectively.

Susceptibility status of *Aedes aegypti* to cyfluthrin and permethrin in Atlantico, Colombia

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The use of insecticides for the control of dengue fever (DF) in the Department of Atlántico for more

than 3 decades has generated the appearance of resistant populations to temephos, fenitrothion, lambda-cyhalothrin, and DDT. It is necessary to evaluate other insecticides as an alternative for the chemical control of the disease. The objective was to evaluate the susceptibility status to cyfluthrin and permethrin in 4 populations of *Aedes aegypti*. Bottle bioassays were performed following the Centers for Disease Control and Prevention methodology, using diagnostic doses for cyfluthrin (25 µg/ml) and permethrin (21.5 µg/ml) against *Ae. aegypti* (F1) from the municipalities of Soledad, Puerto Colombia, and Juan de Acosta in the Department of Atlántico during 2009. Three repetitions, each with 4 replicates and 1 control, were carried out. Susceptibility to cyfluthrin and permethrin was registered in the evaluated populations, showing 100% mortality. The use of insecticides cyfluthrin and permethrin is recommended as an alternative control measure for dengue fever in Atlántico, Colombia.

Enzymes associated with pyrethroid resistance in *Aedes aegypti* (L.) from Veracruz, Mexico

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We used existing biochemical assays to identify and document mechanisms of resistance of 7 populations of *Aedes aegypti* from Veracruz, Mexico. Baseline information on susceptibility of *Ae. aegypti* showed resistance ratios from 10× to 675× to d-phenothrin, permethrin, deltamethrin, lambda-cyhalothrin, bifenthrin, cypermethrin, alpha-cypermethrin, and z-cypermethrin. Results indicate that esterases (alpha and beta) are the main resistance mechanisms in pyrethroid-selected populations, and, less frequently, glutathione-S-transferase and oxidases.

Evaluation of a paint with microencapsulated insecticides for the control of vectors and arachnids

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Vectors and arachnids nest in houses, and improving the house can help control them. For this reason, we evaluated a paint with microencapsulated insecticides (Chlorpyrifos 1.5%, Diazinon 1.5%, and Pyriproxifen 0.063%). We measured the effect on *Triatoma palidipennis* Stal and the scorpion *Centuroides limpidus* Karsch in houses in a rural community of 84 houses from June 2007 to December 2008. Two localities were selected as controls: one having paint without insecticides and the other with nothing. We evaluated the house infestations with 2 biological susceptibility tests on painted walls. After 18 months of follow-up, the houses painted with paint containing insecticides remained uninfested, and the community expressed its appreciation for not having any scorpion bites, while the control houses remained infested and had no change in their infestation levels. The effect of the infestation was statistically significant when analyzed with ANOVA and the Student's *t*-test.

Prevention and control of dengue through community participation

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The design of a good strategy to control and prevent dengue must include community participation. We accomplished this objective through communication strategies and social movement and by evaluating community members' knowledge, attitudes, and practices related to dengue. The methodology employed was as followed: selection of 2 communities from Aragua State, Sector 4, Caña de Azúcar, Municipio Mario Briceño Iragorry (MBI) and Parcela 28, Santa Inés, Municipio Francisco Linares Alcántara (FLA). Then, we established a social network among different institutions related to the communities (community councils, community leaders, educational institutions, universities, Alcaldías, Juntas Parroquiales, others) and health institutions of Estado Aragua. Surveys were conducted on knowledge, attitudes, and practices in selected houses by simple random sampling (50% of houses in each community), interviews, and focus groups. In addition, we looked for likely mosquito oviposition sites and their posi-

tivity and identified other risk factors for their presence (participant observation). We worked to promote dengue prevention from community participation through fundamental values related to axiology, collective, commitment, accountability, and participation. As a result of working 3 months in FLA, there was a remarkable reduction (from 25% to 0%) in mosquito sites (egg, larva, and/or pupa) around the homes. Financial support: Misión Ciencias, Proyecto 2008000911-1, FONACIT-Venezuela.

Barriers to infection and immune response in *Aedes aegypti* during oral infection with dengue

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Transmission of dengue virus depends on the existence of competent mosquitoes, which is determined by multiple factors such as barriers to pathogen development and mosquito immune response. In order to evaluate these aspects, female *Aedes aegypti* from Aragua State were experimentally infected with a strain of DEN-2. Then, the presence of virus in different mosquito body parts was determined. The expression of the defensin gene, involved in innate immune response, in the mosquito was also determined. Results show that the studied population was more susceptible than the reference population (Rockefeller strain); therefore, barriers to infection are easily surpassed by DEN-2. Indeed, females of Aragua population had higher values of experimental parameters for determining infection rate than the Rockefeller strain: minimum infection rate (TMI = 10.2 vs. 7.1), minimum rate dissemination (TMD = 11.4 vs. 5.7), and transmission rate (TMT = 8.5 vs. 5.7). The defensin gene was expressed in mosquitoes fed with a suspension of erythrocytes 4 times more than mosquitoes fed with a similar suspension with DEN-2 virus. These data suggest the existence of a mechanism of viral interference that may allow the persistence of the virus in its vector, favoring enhanced susceptibility to DEN-2.

Effect of physical variables on vertical transmission of dengue virus in *Aedes* mosquitoes from Socunusco, Chiapas, Mexico

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Aedes mosquitoes transmit transovarially all dengue viruses (DENV). Under laboratory conditions, the extent of vertical transmission appears to depend on ambient humidity and temperature. In this study, we determined the effect of relative humidity and room temperature on the rate of DENV vertical transmission in successive generations of *Aedes agypti* and *Ae. albopictus* (Skuse) originated from eggs and larvae collected in Tapachula city, in the state of Chiapas, Mexico. Eggs and larvae were reared to adults under temperature conditions that ranged from 25.35 C to 28.33 C and relative humidity of 47.5–78.4% (SD 2.34–1.52). The presence of DENV in *Aedes* mosquitoes was evidenced by reverse transcription polymerase chain reaction (RT-PCR) of total ribonucleic acid (RNA) extracted from mosquito heads. Several generations of mosquitoes were analyzed in this way. Humidity influenced the vertical transmission of DENV-1, DENV-2, and DENV-3 in *Ae. Aegypti*, and DEN-1 and DEN-3 in *Ae. albopictus* in F0 generation. After this, only DENV-1 was detected in F1 progeny of *Ae. aegypti*. Room temperature seemed not to have an influence on transovarial transmission. The effect of relative humidity on the vertical transmission rate on *Aedes* mosquitoes collected in Tapachula city demonstrates that physical variables have an effect on the reduction of transovarial transmission in successive generations.

Recombinants in dengue virus, serotype-2 isolates, from patients from Oaxaca, Mexico

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Dengue is a serious cause of mortality and morbidity in the world, including Mexico, where the infection is endemic. One of the states with the highest rate of dengue cases is Oaxaca. The cause of dengue is a positive-sense RNA virus that evolves rapidly, increasing its variability due to the absence of a repair mechanism that leads to approximately one mutational event per genome replication, resulting in enhancement of viral adaptation and escape from host immune responses. In addition, recombination may play a role in driving the evolution of dengue virus (DENV), which may potentially affect virulence and cause host tropism changes. Recombination in DENV has not been

described in Mexican strains, and neither has the relevance in virus evolution been described. To study whether there are isolates from Oaxaca characterized by recombination, we obtained the sequence of 6 different isolates of DENV-2 from the outbreak 2005–06, 1 clone of the C(91)-prM-E-NS1(2400) structural genes, and 10 clones of the E gene from 1 isolate. Evidence of recombination was found by using different methods along with 2 software programs: RDP3 and GARD. This is the first report of recombination in DENV-2 in Mexico. Genomic recombinations may play a significant role in DENV evolution and must be considered as a potentially important mechanism for generating genetic variation in this virus with serious implications for vaccines and drug formulations, as occurs for other viruses like poliovirus, influenza, and HIV.

US Environmental Protection Agency's perspectives on efficacy testing for skin-applied repellents and repellent/insecticide-treated textiles

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This presentation provides an overview of US Environmental Protection Agency (EPA) testing guidance for insect repellents. This overview describes the types and breadth of efficacy testing while briefly describing some generic repellent registration requirements. Next, US EPA skin-applied laboratory- and field-testing guidance is discussed and compared to the World Health Organization Pesticide Evaluation Scheme (WHOPES) guidelines. A discussion on testing for repellent/insecticide- treated textiles, such as bed nets, clothing, upholstery, curtains, and wall coverings follows. Comparisons are made to WHOPES guidance and testing requirements from Canada, the US military, the European Union, and at least 1 Latin American nation. Current questions and challenges to conducting human subject testing are included.

US Environmental Protection Agency's perspectives on household and space spray insecticide efficacy testing

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Household insecticides and space sprays are explained in US EPA terms and briefly contrasted to the World Health Organization Pesticide Evaluation Scheme (WHOPES) guidelines. Specifically, the discussion focuses on the approaches the EPA uses to evaluate aerosols (flying insect killers [FIKs]), residual liquids, including crawling insect killers (CIKs), spatial repellents, and ground-applied mosquito space sprays. The presentation also makes comparisons with recently developed testing guidance from the European Union and existing requirements from a few Latin American nations. Some vision/outlook on novel approaches to dengue vector control are provided.

Population dynamics of *Culex quinquefasciatus* in Bogota, Colombia

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Culex quinquefasciatus Say became a nuisance for almost 3 million people living in the south of Bogotá. A study was carried out from December 2006 to December 2007 with the purpose of describing the principal oviposition sites and population dynamics. Excavation pits flooded with contaminated water, small streams, and temporal breeding places were examined. Mosquitoes were collected using modified Shannon and Centers for Diease Control and Prevention (CDC) traps. The most important places found were excavations pits left from construction material factories in the area. These pits were an average of 10 ha in size. The Tunjelito River connects with the pits, filling them with highly polluted water. The density of larvae and pupae of *Cx. quinquefasciatus* was in general influenced by the rain. The biting activity of *Cx. quinquefasciatus* had 2 clear peaks at 1800–1900 h and at 0200–0300 h. During the first peak, nulliparous and multiparous females were found in similar proportion, but in the second peak, mainly multiparous females were found. The highest density was observed after the first rainy season of the year. Although this mosquito species is not transmitting any disease in Bogota, the nuisance has became a public health problem for the community affected because of the mosquito density and bite allergies found in the community, particularly among children.

Malaria entomological inoculation rates in three regions in Colombia

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In Latin America, few attempts have been made to estimate the intensity of transmission using entomological inoculation rates (EIR). A study was carried out in Colombia in 3 different regions between 2007 and 2009 to estimate the EIR. Mosquitoes were collected in a northern part of Colombia, State of Guajira, in the eastern plains, State of Meta, and in the south, State of Putumayo. The majority of the specimens were analyzed by enzyme-linked immunosorbent assay (ELISA) for infectivity. In Guajira, a malaria outbreak took place during 2008, and in total, 1,408 mosquitoes were collected. *Anopheles albimanus* Wieldeman and *Anopheles aquasalis* Curry were found on the coast, while *Anopheles darlingi* Root was found to be the most abundant species inland. *Anopheles darlingi* was found to be positive for *Plasmodium vivax* Feletti and Grassi, and the EIR calculated was 35 infective bites per person per year. In Meta, 3,666 mosquitoes were collected, and *An. darlingi* was also the most abundant species and additionally found positive for *Plasmodium falciparum* Welch. The EIR calculated in this region was 2.71 infective bites per person per year. In southern Colombia, 5,886 mosquitoes were collected. *Anopheles darlingi* was found only in the border with the Amazon state. *Anopheles benarochi (B)* Gobaldon was the most abundant species and was found to be positive for *P. vivax*. The EIR was 98.2 infective bites per person per year. The new species in southern Colombia is maintaining transmission with intensity higher than *An. darlingi*.

First record of urban malaria in Puerto Gaitán, Meta, Colombia

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Puerto Gaitán is located in the northeastern part of Meta State. The internal armed conflict, in addition to false expectations generated by oil exploitation in Puerto Gaitán, has increased the number of displaced persons. As a consequence, during the last year, this municipality, an urban area, reported *Plasmodium falciparum*, *Plasmodium vivax*, and mixed malaria cases. Adult mosquitoes were collected using human landing catches indoors and outside dwellings between 1800 and 0600 h for 50 min/h for 2 consecutive nights per month for 9 months. The oviposition sites were inspected, and the malaria cases per month were analyzed. *Anopheles darlingi* was the most abundant species collected, followed by *Anopheles braziliensis* Chagas, *Anopheles rangeli* Gobaldon, Cova, and Lopez, *Anopheles marajoara* Galvao and Damasceno, and *Anopheles peryassui* Dyar and Knab. *Anopheles darlingi* specimens were caught throughout the night, with 2 peaks of biting activity: indoors (1800–1900 h, 2300–2400 h) and outdoors (2300–2400 h, 0200–0300 h). The main anopheline oviposition sites were Manacacias River overflow and artificial fish ponds. There was no correlation between adult and larval density. We found immature forms from *An. rangeli* and *Anopheles oswaldoi* Peryasu, but adults of these species were not recorded. Malaria control strategies should be focused on adequate diagnostics and immediate treatment for all people, complemented by long-lasting insecticidal nets. In addition, use of personal protection measures to prevent human–vector contact during the first *An. darlingi* peak activity should be promoted. For oviposition sites, keeping the edges of the fish ponds free of vegetation and increasing production of fish that prey on the anopheline immature forms should be promoted.

Evaluation of Mosquito Magnet® to collect malaria vectors in southern Venezuela

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In entomological studies on malaria transmission and evaluation of vector control programs, it is necessary to estimate the human biting rate (i.e., the number of female mosquitoes per person per night). There are ethical and practical reasons for wishing to eliminate or minimize the use of human landing catches. In order to evaluate the efficiency of the trap Mosquito Magnet® (American Biophysics Corporation, North Kingstown, RI) in relation to human landing catches to collect anophelines, a longitudinal study was conducted between June 2008 and January 2009 in Jabillal, a location along the Caura River, Municipality of Sucre, Bolívar State, in southern Venezuela. The village has 37 houses and 101 inhabitants, and malaria is mainly due to *Plasmodium vivax*, with an Annual Parasite Index

of 633.7 per 1,000 population. Two Mosquito Magnet® traps were placed 200 m apart, and 2 human baits were located about 150 m from one of the Mosquito Magnet traps. Collections were conducted from 1730 h until 2130 h, 3 nights per month every 2 months. In total, 1,297 anophelines belonging to 6 species were collected on human landing catches, while the 2 traps caught a total of 597 anophelines representing 7 species. *Anopheles darlingi*, *Anopheles nuneztovari* Gobaldon, and *An. marajoara* were the most common species collected. The analysis of data showed that the number of mosquito of each species collected depended on the method (chi-square = 78.304, $P < 0.05$). To quantify the efficiency of the Mosquito Magnet trap catches compared with human landing catches, the ratios were calculated by dividing the mean of Mosquito Magnet catches by the mean of human landing catches for each of the 3 most abundant species. Confidence limits (CI) were calculated based on the variances of the ratios over different months. The Mosquito Magnet trap was more efficient at collecting *An. nuneztovari* (63%, CI: 2.53), followed by *An. marajoara* (35%, CI: 2.02) and *An. darlingi* (31%, CI: 1.57). There was signifcant correlation between the 2 methods only for *An. darlingi* ($R^2 = 0.48$, $P = 0.004$); the lack of correlation for *An. nuneztovari* ($R^2 = 0.08$, $P = 0.318$) and *An. marajoara* ($R^2 = 0.04$, $P = 0.45$) probably was due to the low abundance and not to the trap itself. Funded by IDRC #103696-006.

Density variation and peridomiciliary hourly biting activity of *Anopheles albimanus* obtained with 2 chemical attractants in Mosquito Magnet Traps, Pacific littoral of Narino, Colombia

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In a field study at the location of La Ensenada (Colombian Pacific littoral), we compared the density and peridomiciliary biting activity of *Anopheles albimanus* lured with 2 commercial baits (MM-Octenol + CO_2 and MM-Lurex3TM + CO_2) in Mosquito Magnet Liberty traps (MM) and human bait–Shannon (HB-S). Captures were carried out for 6 days between 1800 and 0600 h. From a total of 10,210 captured specimens, 57.16% were collected in MM-Octenol, followed by MM-Lurex (24.18%) and HB-S (18.56%). The MM-Octenol traps demonstrated more capture efficiency (972.7 mosquitoes per night) than the other 2 attractants ($P < 0.01$). MM-Lurex and HB-S did not show any significant differences ($P > 0.05$). The hourly MM-Octenol collection showed peaks of biting activity in most of the studied nights, somewhat similar to those obtained with human bait, as described in the literature. However, this similarity is not present in the HB-S of this study. On average, with the 3 attractants, 54.6% to 58.9% of the hematophage activity was focused during the night period (2100–0300 h). The use of the MM-Octenol traps is highly advisable as a sampling and measuring method for biting activity of *An. albimanus*.

Culex and *Coquillettidia* as vectors of West Nile virus in the South American continent

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The objective of this study was to detect the presence of West Nile virus (WNV) in mosquitoes in the northeastern region of Venezuela. Research was conducted in the Laguna de los Patos, Cumana, Sucre State, and the localities of Cicapro (fundo "El Pinal") and Lake Unare, Anzoátegui State, seeking WNV RNA in mosquitoes caught in light traps + CO_2 by reverse transcriptase–polymerase chain reaction (RT-PCR) during the period July 2007 to February 2009. We found a positive mosquito pool of *Coquillettidia venezuelensis* (Theobald) and another pool of *Culex declarator* (Dyar and Knab). The minimum infection rate (MIR) for general copies that year was 0.06, and the minimum infection rate for species would be 0.16 for *Cq. venezuelensis* and 15.8 for *Cx. declarator* for that year. The WNV is active in Venezuela and joins other emerging vectorborne diseases that pose a challenge to research and prevention programs.

Analysis of genetic variation of *Culex pipiens* complex in Mexico City using Ace 2 gene and prediction of the transmission of West Nile virus

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West Nile virus (WNV) was initially isolated in America from species of *Culex* mosquitoes and birds in New York City. Subsequently, the virus spread in the US, and many human cases were reported. *Culex* mosquitoes are considered to be the most important vectors for WNV. The intensity of WNV transmission varies enormously across both space and time, as well as among host species. The causes of this variability are unknown, which makes effective control difficult. In Mexico, this virus was initially detected in equines and birds in Monterrey, Tamaulipas, Coahuila, and Yucatan. Presently, WNV infection is a health problem in the north and south of the country. Consequently, the aim of this research was to investigate the *Culex* species distribution in Mexico City in order to understand the potential of an epidemic of WNV in Mexico. In total, 105 sites were sampled during 2004 for mosquito larva and adult stages. *Culex quinquefaciatus* was the dominant subspecies collected. The importance of this species prompted us to evaluate the oviposition sites of *Culex* species in Mexico. This research also focused on the development of microchips to detect genetic markers useful to differentiate members of the *Culex* complex, the hybrids, and other species using the Ace 2 gene. We determined the genetic variations related to the phenotype in the mosquitoes and the theoretical susceptibility to WNV infection considering the bird migration flyways. The presence of hybrid mosquitoes was confirmed in Mexico City by molecular methods.

Status of insecticide resistance in natural populations from *Aedes aegypti* and KAP surveys of dengue vector in Casanare, Colombia

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Casanare is located in the Llanos Orientales region, an area of endemic dengue transmission. Seven field populations of *Aedes aegypti* were tested using the CDC bioassay methodology for pyrethroid insecticides (lambda-cyhalothrin, deltamethrin, cyfluthrin, permethrin); organophosphate insecticides (malathion, fenitrothion); and the organochlorine DDT insecticide. Temephos insecticide was tested only in larvae using the WHO 1981 bioassay technique. Study sites were selected based upon mosquito infestation index, dengue transmission, and strong vector control activities. The localities were: Yopal (3), Aguazul (2), and Villanueva (2). We carried out 409 house surveys to ask about insecticide control activities and knowledge about the vector in the community. The people said they often used household insecticides. All populations tested showed physiological resistance to DDT insecticide, and lambda-cyhalothrin, and permethrin insecticides. All populations were susceptible to all organophosphate insecticides tested. Although pyrethroids have been recently used for dengue vector control, the insecticide resistance was associated with household and agricultural insecticides applied in these localities. These data provide the initial baselines for insecticide susceptibility profiles for *Ae. aegypti* in the Casanare area.

Low transmission of malaria in the Ecuadorian Amazon Basin: One step closer to eradication

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The Amazon Basin of Ecuador is a hypoendemic area of malaria, where low numbers of cases grouped in sporadic outbreaks make it difficult to measure the transmission of the disease. In total, 839 filter-paper blood samples from 9 villages were tested through indirect ELISA with 4 antigens (*Plasmodium falciparum* MSP-119/AMA1, *P. vivax* MSP119/PvAMA1). Adult specimens surveyed by CDC light/UV traps as well as larvae collected in flooded areas were identified using taxonomic keys and PCR-restriction fragment length polymorphism of the ITS2 gene. Seroprevalence in each community showed areas with high (Santa Rosa PfMSP119: 11%; PvMSP119: 20%; PfAMA119: 17.3%; PvAMA1: 18.2%), medium (Eno: PfMSP119 and PfAMA1: 0%; PfAMA1: 3.5%; PvAMA1: 1.7%), and low seropositivity (Cofan Dureno 0% for all antigens). The entomological study analyzing 192 larvae and adult specimens indicated an inverse relationship between levels of seropositivity and abundance of *Anopheles* (abundance parameter [λ] Santa Rosa = 2.4; Eno = 5; and Cofan Dureno = 9.6; $p < 0.005$). Fifty-two percent of the specimens identified were *Anopheles rangeli*; 16% *Anopheles triannulatus* Neiva and Pinto; 9.9% *Anopheles benarrochi*, and 2.1% *An. oswaldoi*. Serological markers proved to be a useful tool to assess malaria transmission in hypoendemic areas. Low density of mosquitoes and unstable transmission of malaria suggest an interesting perspective for

elimination and eradication of the disease. This is the first report of anopheline species at a molecular level in the region.

Status of insecticide resistance in *Anopheles aquasalis* in two malaria-endemic regions in Venezuela

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Anopheles aquasalis is the main vector of malaria in coastal regions of Venezuela. Resistance to organic insecticides was characterized in adult mosquitoes in the states of Sucre and Delta Amacuro. This was done by evaluating the expression of resistance to the insecticides fenitrothion and lambda-cyhalothrin, which are used for mosquito control in these states, using the bottle methodology developed bythe US Centers for Disease Control and Prevention. We also identified metabolic resistance mechanisms when they were compared with a reference strain from Brazil by testing in microplates and polyacrylamide gel electrophoresis. The results indicate that mosquitoes from Delta Amacuro were resistant to both insecticides, while in Sucre, they were susceptible to lambda-cyhalothrin but resistant to fenitrothion. Both strains showed synergism with piperonyl butoxide, suggesting resistance mechanisms based on enzymes. We identified insensitive acetylcholinesterase and alpha and beta esterases; the mixed-function oxidases were elevated in Sucre (0.5), while glutathione-S-transferase was not detected. The knowledge gained contributes to the improvement of strategies within regional malaria vector control programs.

Sentinel surveillance of mosquitoes using larvitraps in the Department of Atlantico, Colombia, 2004–08

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The Department of Atlántico presents risk factors for the transmission of vectorborne diseases. The objective was to report the Culicidae species recorded using an entomological system of surveillance based on larvitraps located in urban and periurban areas in the Department of Atlántico, Colombia, between 2004 and 2008. We obtained weekly information of the entomological material collected in 14 larvitraps located in the international airport (2004–08) and in 5 larvitraps placed in the transport terminal (2007–08) in the municipality of Soledad and in 10 larvitraps settled in the army battalion quareters (2005–08) in the municipality of Malambo. We collected 83,369 mosquito larvae : 37,370 (45%) from the international airport, 35,376 (42%) from the transport terminal, and 10,623 (13%) from the army battalion. *Aedes aegypti* was the most abundant species (89.21%), followed by *Culex nigripalpus* Theobald (3.45%), *Cx. quinquefasciatus* (2.46%), *Haemagogus equinus* Theobald (2.25%), *Culex coronator* (1.19%), *Uranotaenia lowii* Puzos (0.91%), *Culex stigmatosoma* Dyar (0.52%), and *Aedes taeniorhynchus* Wiedeman, *Anopheles albimanus*, and *Toxorhynchites* spp. with 0.01%, respectively. The presence of vector species was confirmed in urban and periurban areas of the Department of Atlántico. It is necessary to maintain a permanent entomological surveillance system in order to take prevention and control measures in time.

Presence of *Anopheles* (*Kerteszia*) *pholidotus* in a malaria focus in Colombia

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In the Colombian malaria study at Cunday-Villarrica in Tolima, *Anopheles* (*Kerteszia*) *lepidotus* (Zavortink) was incriminated as the main vector in 1984 because of their almost exclusive presence in most of the localities with malaria transmission. Since then, this species has been included, along with 6 other species, as one of the malaria vectors in Colombia. Between February and August 2009, collections were carried out in the same localities as the 1984 study as part of a study to determine biological aspects of the anopheline species of subgenus *Kerteszia* in Colombia. Approximately 800 adult females of

putative *An. lepidotus* designation were collected landing on humans, and 37 series of larvae from bromeliads were reared. Male genitalia were mounted and compared with the morphological keys and descriptions available for *Kerteszia* species. The morphological characteristics of all male genitalia corresponded with the descriptions and keys for *Anopheles pholidotus* (Zavortink). It is suggested that the incriminated malaria vector species in Tolima in 1984 corresponds to *An. pholidotus* and not *An. lepidotus*. However, confirmation of the taxonomic determination using molecular markers is necessary.

Anopheles (*Nyssorhynchus*) *strodei*, a species complex in the subgenus *Nyssorhynchus* of *Anopheles*

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Nyssorhynchus is one of the most studied subgenuses of the Neotropical *Anopheles*; however, some species still are poorly known. *Anopheles strodei* Root includes 5 species in the synonymy, *Anopheles ramosi*, *Anopheles arthuri*, *Anopheles artigasi*, and *Anopheles albertoi*, described from Brazil, and *Anopheles lloydi*, from Panama. Morphological characteristics of the eggs, adult male and female, and sequence data of the mitochondrial cytochrome oxidase subunit I (COI), nuclear white gene, and ITS2 ribosomal deoxyribonucleic acid (DNA) from individuals collected in several localities situated in the states of Espírito Santo, Minas Gerais, São Paulo, Paraná, and Rondônia, including the type localities of *An. strodei* and also its synonymies, corroborate that there are at least 4 valid species under the name *An. strodei* in Brazil, and thus some of its junior synonymies are valid species.

Distribution of *Anopheles darlingi* lineages in Colombia

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Anopheles darlingi is the main malaria vector in Latin America. It has been proposed that this species consists of 2 lineages. The northern lineage was found in populations from Central America and in 1 population from the northwest of Colombia (Nechi, Antioquia). The southern lineage was found in Amazonia (Brazil, Peru, and French Guiana). The purpose of this study was to determine the lineages present along the distribution range of *An. darlingi* in Colombia. Mosquitoes were collected in the northern part of Colombia in La Guajira, the eastern plains in the state of Meta, and in the south in the Amazonian state of Putumayo. In total, 49 sequences were obtained for the single copy nuclear DNA white gene and 48 for a fragment of the mtDNA COI gene. A statistical parsimony network demonstrates that both lineages are present in Colombia. Both data sets suggest that individuals from La Guajira belong to a group that corresponds to the northern lineage, and the Putumayo and Meta populations correspond to the southern lineage. We hypothesize that the Andes Mountains played an important role in the differentiation of these lineages.

Comparison of genetic diversity among different Latin American populations of *Aedes aegypti*

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Dengue is one of the most important viral diseases in Venezuela transmitted by arthropod vectors. The genetic diversity of the vector determines its susceptibility to viral infection, and that is why it is necessary to be aware of the introduction of possible new strains in a country. Therefore, we decided to study the genetic diversity of *Aedes aegypti* from different countries of Latin America to compare it with the one from Venezuela. For rapid characterization, variation in a 387-base-pair region of the nicotinamide adenine dinucleotide dehydrogenase subunit 4 mitochondrial gene (ND4) was determined by single strand confirmation polymorphism analysis. Preliminary analyses of mosquitoes collected (~50/country) from Argentina, Brazil, Colombia, Mexico, Puerto Rico, and Peru indicate the presence of 1 or 2 haplotypes per each country's sample. Some haplotypes have similar sizes, ranging between 1,000 and 1,500 base pairs; however, sequencing of DNA amplicons is needed to identify haplotypes. Argentina, Mexico, and Peru only have one haplotype; Colombia, Brazil, and Puerto Rico have 2. The frequencies of the

principal haplotypes are: 96.2% (Colombia), 81.1% (Brazil), and 58.2% (Puerto Rico). These results indicate that *Ae. aegypti* populations from these countries have a low level of genetic diversity; 3 of them are monomorphic. This suggests that the mosquitoes in all countries are under strong selective pressure.

Use of the barcode region for the identification of species belonging to *Albitarsis* Group (Linthicum)

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The *Albitarsis* complex is formed by six species: *Anopheles albitarsis* Lynch-Arribálzaga, *Anopheles albitarsis* F. (Brochero et al. 2007), *Anopheles deaneorum* Rosa-Freitas, *Anopheles janconnae* Wilkerson and Sallum, *An. marajoara*, and *Anopheles oryzalimnetes* Wilkerson and Motoki. This complex has great importance in malaria transmission; *An. marajoara* and *An. janconnae* (Povoa et al. 2006) have been incriminated as vectors in Brazil, displacing *An. darlingi* in some areas (Conn et al. 2002). Their role as malaria vectors in other countries is unknown due to taxonomic ambiguities. The barcode region (COI, 710 bp) was postulated by Hebert et al. (2003) as a DNA sequence–based method for the accurate identification of species, even in cryptic species complexes. Only 2 studies have been reported using this region for mosquito identification: Cywinska et al. (2006) and Kumar et al. (2007) for Canadian and Indian mosquitoes, respectively. In the present research, 479 mosquitoes were sequenced from Argentina, Brazil, Colombia, Paraguay, Trinidad and Tobago, and Venezuela, resulting in 281 haplotypes, forming 6 phylogroups (taxa) using K2P: *An. albitarsis*, *An. deaneorum*, *An. janconnae*, *An. marajoara* (taxon 1 and 2), and *An. oryzalimnetes*. Preliminary results confirm this methodology as a useful tool for molecular identification of some species formally described belonging to the *Albitarsis* Group: *An. albitarsis*, *An. deaneorum*, *An. janconnae*, and *An. oryzalimnetes*. However, caution should be used with the identification of *An. marajoara*, which may be misidentified as *An. albitarsis* F. and other unknown species in the *Albitarsis* Group.

Blood-feeding insects from a high-altitude region in Colombia

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Information about the biodiversity of hematophagous insects from high altitudes is very scarce. This fauna is characterized by its high endemism. The objective of this study was to report Ceratopogonidae and Simuliidae species using different trapping methods in Chingaza National Natural Park, Colombia. This park has 76,000 ha and is the source of several rivers. Most of the area is located above 3,000 m above sea level. The temperature fluctuates between 2 °C and 14 °C during the year. The following traps were used to capture insects: CDC light trap, CDC modified trap (light and CO_2), Shannon modified trap, Malaise trap, swab net, and human and animal protected bait. These traps were located close to the streams. Immature stages of Simuliidae were collected on stones and overflowing vegetation in streams. In total, 582 specimens of *Culicoides*, *Culicoides suarezi* Rodriguez and Wirth, and *Culicoides* sp. nov., and 1 *Simulium furcillatum* Wygodzinsky and Coscaron were trapped. Most of the *Culicoides* (524 specimens) and the black flies were collected with human protected bait. Immature stages, larvae, and pupae of Simuliidae (>1,000 specimens) were captured on stones and overflowing vegetation in streams. These results show that *C. suarezi* and *C.* sp. nov from Chingaza Natural National Park have anthropophilic habits.

Mosquito vectors of human diseases related to Phytotelmata dwelling places in Colombia

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Phytotelmata dwelling places are important breeding places of mosquito vectors. Nevertheless, the study of these dwelling places in Colombia has been limited. The purpose of

this study was to identify the mosquitoes existing in *Guadua* and *Bromelia*, and to characterize the importance of these places. The study areas were located in Antioquia, Caldas, and Chocó. Larvae were collected from the phytotelmata dwelling places, and adult mosquitoes were caught with protected human bait. Series of mosquitoes were examined. Exuviaes, larvae, and adult mosquitoes were molecularly and/or morphologically identified. The results show the presence of *Anopheles eiseni* Coquillett, *Culex antunesi* Lane and Whitman, *Culex secundus* Bonne-Webster and Bonne, *Orthopodomyia albicosta* Lutz, *Limatus durhamii* Theobald, *Trichoprosopon digitatum* Rondani, *Trichoprosopon* sp., *Sabethes undosus* Coquillett, *Wyeomyia oblita* Lutz, and *Toxorhynchites* sp. in *Guadua*. Bromeliads found were *Phoniomyia longirostris* Theobald, *Ochlerotatus* sp., *Culex* sp., *Wyeomyia* sp., *Sabethes* sp., *Anopheles neivai* Howard, *Trichoprosopon* spp., and *Anopheles* sp. The specific confirmation is being made by the Centers for Diseases Control and Prevention in Atlanta. These findings contribute to the ecological and medical acknowledge of the mosquito fauna in Colombia. In addition, this is part of an international initiative for the assignment of a genetic barcode for mosquitoes, led by the London Natural History Museum, with the participation of Antioquia University and National University.

Resistance profile to pyrethroids in *Aedes aegypti* from the east coast of Mexico

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Pyrethroid resistance of 7 field strains of *Aedes aegypti* adult females from Veracruz, Mexico, to d-phenothrin, permethrin, deltamethrin, lambda-cyhalothrin, bifenthrin, cypermethrin, alpha-cypermethrin, and z-cypermethrin was investigated and compared with a susceptible strain (New Orleans) by using the bottle bioassay. Knockdown resistances (KD) after 1 h of exposure (RRKD-50) and resistance after 24 h (RRLD-50) of recovery were calculated. Cluster analysis of the KD50s for each of the 8 compounds indicated that the knockdown resistance profiles were very similar between d-phenotrin and alpha-cypermethrin, permethrin and cypermethrin, and deltamethrin and lambda-cyalothrin and z-cypermethrin. Resistance profile for bifenthrin was not correlated with any of the other 7 compounds. Regression analysis of RRLD-50 and RRKD-50 indicated that more than 8 times the amount of lambda-cyhalothrin is required to cause lethality versus knockdown, 2.68× more is required for z-cypermethrin, and amounts for the others are as follows: 2.63× for deltamethrin, 2.40× for alpha-cypermethrin, 1.91× for permethrin, 1.89× for cypermethrin, 1.66× for biphentrin, and 0.875× for d-phenothrin.

Altosid XRG against *Aedes aegypti* in laboratory and outdoor conditions

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Effectiveness and residual effect tests of a granular formulation of an insect growth regulator against laboratory-reared larvae of colonized mosquitoes, *Aedes aegypti*, were conducted in laboratory and outdoors. For the laboratory phase, we used 12 stainless-steel containers with a capacity of 5.8 liters, for 6 tests and 6 controls (prepared with water). For the field phase, we used 9 plastic bins with a capacity of 100 liters, 3 of which were used for the product, 3 for controls (water only), and 3 for experimental controls with temephos (commercial product formulation granules). The XRG test dose was 1.5 g product in 100 liters of water. Three days after container treatment, 3rd-stage larvae were added. We used 25 larvae per replicate both in treatment and controls. The larvae were fed balanced fish food (Aqua Crece®) every 2 days during the bioassays. Mortality was recorded daily, and pupae were removed to observe the emergence of adults. We recorded temperature and pH of the containers daily. Altosid XRG showed satisfactory efficiency in the laboratory up to 33 days and diminishing effectiveness after 44 days. The high level of eutrophication in the test trays can be a condition limiting the effectiveness of the product. For field tests, the product showed satisfactory efficiency after 50 days.

Use of *Metarhizium anisopliae* for control of *Aedes aegypti* adults

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